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Combined effects of temperature and salinity on the physiology of two geographically-distant eastern oyster populations

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ABSTRACT

Understanding why a species occupies a certain geographic range and predicting how they will be affected by climate change require characterizing physiological traits in geographically-distant populations. The objective of this study was to perform a direct comparison of two eastern oyster (*Crassostrea virginica*) populations that occupy contrasting temperature and salinity habitats, New Brunswick, Canada (47°N – Gulf of St. Lawrence) and Louisiana, USA (29°N – Gulf of Mexico). Specifically, clearance rate, valve opening, and oxygen consumption rate were measured in oysters of both populations following a full factorial design with three temperatures (10, 20, 30 °C) and two salinities (15, 25). New Brunswick oysters had a greater gill area, shell gape angle, and oxygen consumption rate. Temperature was the main driver of clearance rate, valve opening duration and oxygen consumption rate. Clearance rate at 20 °C and 30 °C was significantly higher than at 10 °C, and oysters at 20 °C had their valves opened for a greater percentage of time compared to oysters at 10 °C and 30 °C. Oxygen consumption rate increased gradually from 10 °C to 30 °C and similarly for both populations, with no indications of a thermal breakpoint at 30 °C. Temperature coefficients for the oxygen consumption rate (Q_{10}) were within the normal ecological range for both populations. No physiological differences were observed between salinity treatments in both populations. No latitudinal compensation in the physiological rates was found. Overall, results showed that *C. virginica* is tolerant to a broad range of temperatures and salinities. Such physiological plasticity is consistent with the species' extended geographical range.

1. Introduction

A pattern of intraspecific variation in growth rate along latitudinal gradients has been found in many ectotherm species, with individuals from northern populations growing faster than conspecifics from southern populations when raised under the same conditions (Dehnel, 1955; Conover and Present, 1990; Yamahira and Conover, 2002; Lindgren and Laurila, 2009). Levinton (1983) proposed that those differences evolved in response to adaptation to native temperature regimes such that high-latitude individuals grow faster than low-latitude individuals at low temperatures, but more slowly at high temperatures. Other studies have however demonstrated that high-latitude individuals have higher capacity for growth at both high and low temperatures (Conover and Present, 1990; Yamahira and Conover, 2002) and this latitudinal compensation may be an adaptation to shorter growing seasons. Less is known regarding the underlying molecular, biochemical, and physiological mechanisms and the role that energy allocation (i.e. growth, reproduction) plays in these growth patterns (Angilletta Jr et al., 2004). For example, the metabolic cold adaptation hypothesis states that populations or species from cold environments will have elevated metabolic rates compared to those from warmer waters; however, it has been a topic of debate over many years and some studies have failed to find evidence for latitudinal metabolic compensation (Clarke, 1993; Peck and Conway, 2000; Addo-Bediako et al., 2002).

The eastern oyster *Crassostrea virginica* is an ectotherm and

Abbreviations: CR, Clearance rate; CRi, Individual oyster clearance rate; CRg, Clearance rate standardized by gill area; CRw, Clearance rate standardized by dry meat weight; LA, Louisiana; NB, New Brunswick; OCR, Oxygen consumption rate; OCRi, Individual oxygen consumption rate; OCRw, Oxygen consumption rate standardized by dry meat weight; Q_{10}, Van't Hoff's temperature coefficient

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euryhaline species with a wide latitudinal distribution ranging from the Gulf of St. Lawrence to the Gulf of Mexico and the Caribbean (Galtsoff, 1964; Lazoski et al., 2011). Because of its large geographic extension, oyster populations are exposed to very different thermal and environmental conditions (i.e. food availability, photoperiod, etc.). For example, in the shallow estuaries of the Gulf of St. Lawrence (Canada) water temperature seasonally varies between −1.5 °C and 22 °C, and temperatures above 5 °C, considered to be the lower feeding threshold, occur for about half of the year (Comeau et al., 2012). In contrast, temperature in the northern Gulf of Mexico (USA) varies between 9 °C and 32 °C, a range that is favorable for feeding all year round (Loosanoff, 1958; Casas et al., 2015, 2017; Leonhardt et al., 2017; Lowe et al., 2017). Moreover, genetic differences between Atlantic and Gulf of Mexico *C. virginica* have been repetitively shown (Reeb and Avise, 1990; Murray and Hare, 2006) and there is increasing evidence for the existence of genetic subpopulations (Rose et al., 2006; Varney et al., 2009, 2016). However, there is little information on the comparative physiology of geographically-distant populations. Early studies noted that northern populations begin spawning at a lower temperature than southern populations. For example, oysters from Long Island Sound spawned at temperatures above 16 °C while populations from Galveston Bay required 25 °C (Stauber, 1950; Loosanoff and Nomejko, 1951; Barber et al., 1991). Differences in growth rate between oyster populations across latitudinal clines have also been found, along with higher gill ciliary activity in colder water temperatures in northern populations, supporting the latitudinal compensation hypothesis, since gill ciliary activity is correlated with feeding rate (Dittman, 1997; Dittman et al., 1998).

Recently, macro-physiology studies examining how widely-distributed organisms are affected by the high variability of environmental conditions have arisen to understand and predict how species and ecosystems will respond to climate change (Osovitz and Hofmann, 2007). The need for studies to assess organismal condition and physiological performance across entire species' ranges has therefore increased. Such research, however, is not very common due to the logistical challenges of working at large spatial scales. Jansen et al. (2007) measured the oxygen consumption rates of 16 populations of mussel species (*Mytilus* spp.) distributed along the European coast. Measurements were done at five temperatures (3–31 °C) and at different times of the year. Temperature sensitivity of the oxygen consumption rate was characterized by calculating Q_{10} or van't Hoff's temperature coefficient; Q_{10} values ~ 2 occur when the rate is measured within the ecological temperature range of the species, and Q_{10} values < 1 mean that a breakpoint temperature is reached and respiration rate decreases with increasing temperature (Jansen et al., 2007; Pörtner, 2001; Bayne, 2017). Mean oxygen consumption rates in *Mytilus* spp. increased from 3 °C to 24 °C and then decreased after reaching a breakpoint temperature between 24 °C and 31 °C (Jansen et al., 2007) with Q_{10} < 1 occurring at lower temperatures in northern than southern populations. Interestingly, a breakpoint temperature was reached in summer at one of the southern population location, and was associated with mussel mortalities. Recent studies have documented climate-related mortality events, changes in population abundances, and shifts in the geographical distribution of ectothermic animals (Helmuth et al., 2005; Perry et al., 2005). In species with a wide latitudinal range, climate change will probably have the most deleterious consequences in the southern populations because they are currently living closer to their breakpoint temperature (Pörtner, 2001; Jansen et al., 2007). Regarding *C. virginica* populations, it is largely unknown how climate change will affect their abundance and geographical distribution, and the thermal sensitivity of the oxygen consumption rate of geographically-distant populations has not been compared.

Salinity also affects *C. virginica* physiology and distribution and differences in performance between populations growing under the same conditions has been documented (Powell et al., 1992; Shumway, 1996; Lavaud et al., 2017; Leonhardt et al., 2017). There is surprisingly little information available for the combined effect of temperature and salinity on the physiological rates of geographically-distant oyster populations when temperature and salinity control every aspect of their biology (Shumway, 1996; Bayne, 2017). The objective in this study was to compare the physiological performance of a northern (47°N – Gulf of St. Lawrence) and a southern (29°N – Gulf of Mexico) *C. virginica* populations. Specifically, clearance rate, valve opening, and oxygen consumption were measured in oysters of both populations following a full factorial design in the laboratory with three temperatures (10, 20, 30 °C) and two salinities (15, 25). We tested the hypothesis that physiological performance of distant oyster populations adapted to contrasting thermal and saline environments may differ when tested under a range of temperature and salinity laboratory conditions. This experiment was designed to test latitudinal compensation in the physiological rates of two geographically-distant populations and to provide one of the first insights into performance, physiological plasticity, and thermal tolerance of *C. virginica* populations.

2. Materials and methods

2.1. Oysters

Wild oysters were collected from Baie Saint-Simon-Sud (47.7173°N; 64.7822°W), New Brunswick (NB), Canada and southeast Louisiana (LA), (Bay Gardene [29.5910°N; 89.6425°W] and Sister Lake [29.2341°N; 90.9172°W]), USA. Baie Saint-Simon-Sud is characterized by water salinity ranging from 18 to 31, and temperatures from −1.5 °C to 22 °C (Comeau et al., 2012, 2017), whereas southeast Louisiana is characterized by water salinity ranging from 1 to 28, and temperatures from 9 °C to 32 °C (Casas et al., 2015; Leonhardt et al., 2017; Lowe et al., 2017). Oysters from Canada (Fig. 1) were approximately six years old with a shell height of 71.1 ± 4.9 mm, $N = 50$ (mean ± standard deviation). Oysters collected from southeast Louisiana were maintained at the Louisiana Sea Grant Oyster Research Hatchery and Demonstration Farm (Grand Isle, LA, USA) prior to experimentation and were estimated to be about one and a half years old and 70.3 ± 7.2 mm in shell height, $N = 50$ (Fig. 1). The study was timed at the end of the fall to wait for both populations to finish with their reproduction and be in resting gonadal stage, and to collect the New Brunswick oysters before freezing of the seawater.

2.2. Experimental design

In October 2015, 350 oysters from each population were collected and transported to Louisiana State University. At the time of collection, water temperature and salinity in Baie Saint-Simon-Sud and Grand Isle were 8 °C and 26, and 21 °C and 12, respectively. In the laboratory, oysters of each population were scrubbed and divided into six groups. Each group was placed in a 200–500 L tank filled with artificial seawater (Crystal Sea, Marinemix Marine Enterprises International, Baltimore, Maryland, USA) adjusted to the salinity of the place of collection and 16 °C. Salinity was gradually modified, at a rate of 3 units per day, every other day, until the experimental salinities of 15 and 25 were reached. Next, water temperature in the tanks was gradually

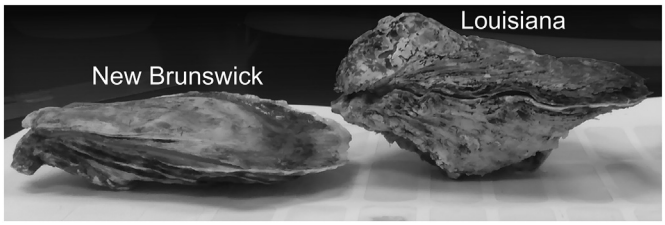

Fig. 1. Photograph illustrating the differences in shape between New Brunswick and Louisiana oysters.

adjusted at a rate of 1–2 °C per day until reaching the experimental temperatures of 10, 20 and 30 °C. A total of 12 tanks were used in the experiment (2 salinities × 3 temperatures × 2 populations), and one tank for each set of conditions. A minimum of 50 oysters were placed within each of the tanks and were available for testing, with the individual oyster representing the unit of measurement. Water quality in the tanks was checked twice weekly to ensure similar conditions between populations. Oysters were maintained at the experimental salinity and temperature combination for a minimum of two weeks prior to the physiological measurements. Oysters were fed daily at approximately 5% of their meat dry weight with Shellfish Diet 1800® (Reed Mariculture Inc., Campbell, CA, USA); a commercial mix of six marine microalgae: *T Isochrysis lutea* (strain CCMP1324), *Pavlova* sp. (strain CCMP359), *Tetraselmis impellucida* (strain CCMP892), *Chaetoceros calcitrans*, *Thalassiosira weissflogii* (strain CCMP1051), and *Thalassiosira pseudonana*. Shellfish Diet 1800® was added to the tanks in one dose at the end of the day. Clearance rate, valve opening, oxygen consumption rate as well as oyster size and weight measurements were completed within a two-week period starting December 1, 2015, following the protocols listed below.

2.3. Clearance rate

Clearance rate (CR), defined as the volume of water completely cleared of suspended particles per unit of time, was quantified in a static system (Widdows, 1985). Individual oysters were placed in 2 L beakers filled with 0.5 µm filtered seawater and left undisturbed for about 60 min. Shellfish Diet1800® was added to each beaker to give an initial concentration of ~ 30,000 cells mL^{-1}. Algae concentration in 25-mL samples collected every 10 min during a 40 min period was measured with a particle size analyzer (Multisizer 3 Beckman Coulter Counter, 100-µm pore orifice, considering only particles ≥ 5 µm). Algal sedimentation was reduced by gentle aeration of the water with air stones. Beakers with closed oysters were not sampled. Beakers containing the same concentration of algal cells but empty shells were used as controls. Individual oyster clearance rate (CRi) was calculated according to the equation $CRi = [(b - b') \times Vol \times 60]$ where b is the slope of the linear regression between the natural logarithm of cell concentration (cells mL^{-1}) and time (min) for the beaker with oyster, b' is the slope for the control beaker, Vol is the volume of seawater in the beaker (2 L), and 60 (min h^{-1}) is used to convert the time units. This method follows Cranford et al. (2016), in which a generalization of Coughlan's method (Coughlan, 1969) is developed to integrate the values of multiple particle concentration samplings over time. This approach aims to reduce the impact of particle counting outliers in clearance rate estimations by including multiple data points instead of relying on initial and final concentration data points solely. Accordingly, only linear regressions with an $r^2 > 0.90$ were included in these calculations (Cranford et al., 2016).

Clearance rate should be expressed relative to gill area to recognize the direct relationship between gill area and clearance rate (Meyhöfer, 1985; Riisgård, 1988) and to avoid standardization errors related to the condition of the animals compared (Filgueira et al., 2008; Cranford et al., 2011). After completing clearance rate measurements, gill areas were measured using digital photography. Oysters were opened, the right shell removed and the edge of the mantle moved away from the gills. The gills were placed flat and parallel to the camera lens, and a picture taken including a ruler to calibrate the image. Didger 3.03 (Golden Software LLC, Golden, Colorado, USA) was used to determine gill areas, which were expressed in cm^2 and used to calculate clearance rate by gill area (CRg) in L h^{-1} cm^{-2}, following Filgueira et al. (2008). In addition, to compare with previous published data on *C. virginica,* clearance rates were standardized to a 1 g dry meat weight according to equation CRw = CRi × (DWstd/DWexp)^b, where CRw is the clearance rate of a standard dry weight animal, CRi is the clearance rate of the experimental animal, DW_{std} is the standardization dry weight (1 g), DW_{exp} is the dry weight of the experimental animal, and *b* is the dry weight exponent (0.58, Cranford et al., 2011).

2.4. Valve opening

A valvometry system previously described in Nagai et al. (2006) and Comeau et al. (2012) was used to monitor valve opening in two to four oysters per treatment ($N = 40$ total). Briefly, for each oyster, a coated Hall element sensor (HW-300a, Asahi Kasei Corp, Chiyoda-ku, Tokyo, Japan) was glued to one valve at the maximum distance from the hinge. Then a small magnet (4.8 mm diameter × 0.8 mm height) was glued to the other valve, directly below the Hall sensor. The magnet and the Hall element weigh 0.1 g and 0.5 g, respectively. The magnetic field (flux density) between the sensor and the magnet is a function of the gap between the two valves. The magnetic field in the form of output voltage (µV) was recorded at a frequency of one minute by dynamic strain recording devices (DC 104R, Tokyo Sokki Kenkyujo Co., Shinagawa-ku, Tokyo, Japan). To allow for acclimation following the gluing of sensors and magnets, the first day of valve monitoring was discarded from the dataset and data analyses were restricted to a three-day period (Dec 2 2015 [10 AM] to Dec 5 [10 AM]). Over the course of the monitoring period, two probes (CYCLOPS-7® submersible sensor, Turner Designs, Sunnyvale, CA, USA) were sequentially deployed in the experimental tanks to record chlorophyll *a* levels (relative fluorescence unit or RFU). At the end of the experiment, the adductor muscle was severed, and small calibration wedges (1–6 mm) were inserted between the two valves at the point farthest from the hinge to derive a voltage versus mm calibration curve ($r^2 > 0.90$) specific to each oyster. Output voltage measurements were then converted into valve opening (mm) data and gape angles (θ in degrees) as described in Wilson et al. (2005). The propensity for valve openness was calculated for each oyster as the percentage of time valves were opened (time opened/[time opened + closed]) and mean gape angle (θ in degrees).

2.5. Oxygen consumption rate

Oxygen consumption rate (OCR) was also estimated in a static system. Individual oysters were placed in 915-mL acrylic chambers filled with seawater from their holding tank. The chambers were closed under water with lids which had an opening for inserting a self-stirring ProOBOD probe (YSI Incorporated, Yellow Springs, OH, USA) with optical dissolved oxygen and temperature sensors. A chamber containing empty shells was used as a control. Each oyster was allowed to settle in the respirometer and readings started once valves opened. Oxygen and temperature readings were automatically stored every 30 s in an YSI Multilab 4010–3 m (YSI incorporated) connected to the probes. The recording period lasted 90 min and was ceased if the percentage of oxygen was below 70% of saturation. Up to 9 respirometry chambers were measured at the same time. Individual oyster oxygen consumption rate (OCRi) was calculated as $OCRi = [(b - b') \times Vol \times 60]$ where b is the slope of the linear regression between the concentration of oxygen (mg L^{-1}) in the chambers with oysters versus time, b' is the slope for the control, Vol is the volume (L) of water in the respirometer (respirometer minus oyster volume), and 60 (min h^{-1}) is used to convert time units. Only linear regressions with an $r^2 > 0.95$ were included in the calculations. Oxygen consumption rates were standardized to an equivalent 1 g dry tissue weight using the formula described above for clearance rate and a weight exponent of 0.58 (Casas et al., 2018) and was designated OCRw.

For measuring the effect of a 10 °C rise in temperature had on the oxygen consumption rate, Q_{10} were calculated following the expression $Q10 = (OCRw_{T2}/OCRw_{T1})^{(10/(T2 - T1))}$, where $OCRw_{T2}$ and $OCRw_{T1}$ are the oxygen consumption rates at the higher and the lower temperatures, T2 and T1, respectively (Jansen et al., 2007).

2.6. Condition index, size, and shell shape

After all physiological measurements were completed oyster volume, shell shape (shell height, width, and length, Galtsoff, 1964) and weights (whole wet oyster, wet and dry shell, and dry meat) were measured. Cup ratio of individual oyster was calculated by dividing shell width by shell height and fan ratio was calculated by dividing shell length by shell height. Condition index (CI) was calculated as the ratio of dry meat weight to the whole wet oyster weight minus its shell wet weight (i.e. filled shell cavity weight) multiplied by 100 (Abbe and Albright, 2003). The condition index is a prime indicator of how well an oyster uses the available internal shell cavity for somatic and gonadal tissue growth and reflects physiological or nutritive status (Haven, 1961; Rainer and Mann, 1992).

2.7. Statistical analysis

SPSS 24 (IBM SPSS Inc., Chicago, USA) was used to analyze all data. Clearance rate (CRi, CRg, CRw), oxygen consumption rate (OCRw), condition index, dry meat weight, and filled shell cavity weight were examined for normality (Shapiro-Wilks test) and homogeneity of variance (Levene's test), transformed as required and analyzed with a three-factor (i.e. temperature, salinity and population) analysis of variance (ANOVA, type III sum of squares) followed by the post-hoc test Hochberg's GT2, which minimizes the effect of an unbalanced number of cases per treatment. Shapiro-Wilks was also applied to the unstandardized residuals of the model to verify normality. Shell height, cup and fan ratio, shell weight to shell height ratio, and gill area were compared with a one-factor (population) ANOVA. Polynomial regressions were used to determine the effects of temperature and salinity on OCRw. All data are presented as mean ± standard deviation.

Regarding valvometry, cross-correlation analyses were performed between gaping and chlorophyll *a* time series. The intent was to assess whether behavior was related to the daily addition of phytoplankton into the experimental tanks. Prior to these analyses the two time series were first-order differenced to remove the presence of autocorrelation (Chatfield, 2004). In addition, three-factor ANOVAs were conducted to test for the main effects of population, temperature, and salinity on the percentage of time valves were opened and on gape angle when valves were opened. Data were rank-transformed to stabilize the variance, and ANOVAs followed by Hochberg's GT2 post-hoc test performed after validating normality (Shapiro-Wilks test) and homogeneity of variance (Levene's test). In the case of the percentage of time oysters were opened, normality within groups was not tested due to the low number of cases in some treatments ($N = 2$). Shapiro-Wilks was applied to the unstandardized residuals of the model to verify normality.

3. Results

3.1. Clearance rate

Clearance rate standardized by gill area (CRg) was significantly affected by the interaction of temperature, salinity, and population (Table 1, Fig. 2, see full ANOVA in Supplementary Table 1). Overall, CRg was significantly lower at 10 °C (0.11–0.35 L h^{-1} cm^{-2}) than at 20 °C (1.04–2.52 L h^{-1} cm^{-2}) and 30 °C (1.36–2.87 L h^{-1} cm^{-2}) for both population and salinity. The three way significant interaction is due to the Louisiana population (10 °C, 15) not differing significantly from the New Brunswick population (20 °C, both salinities). Similarly, the CRg of all 20 °C and 30 °C populations and salinities were not significantly different, except for the Louisiana (30 °C, 25) population which differed only from the New Brunswick (20 °C, 15) population.

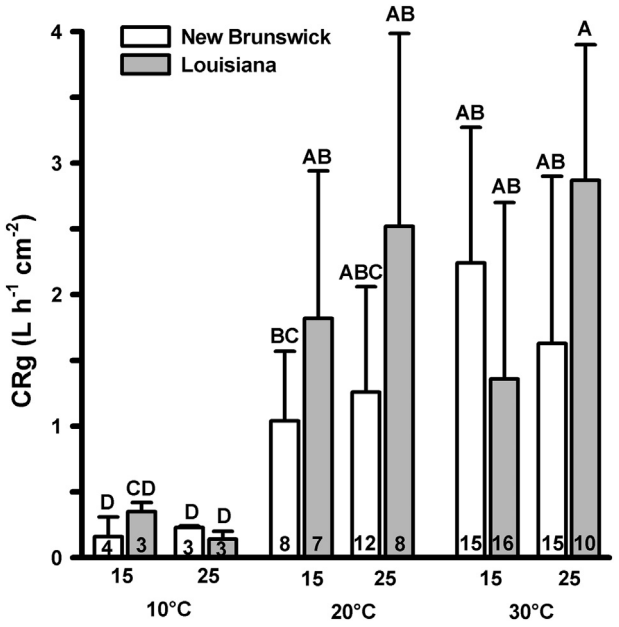

Fig. 2. Combined effects of temperature (10, 20, 30 °C) and salinity (15 and 25) on the clearance rate of two populations of *Crassostrea virginica*. Data are presented as mean clearance rate standardized by gill area (L h^{-1} cm^{-2}) ± standard deviation. Different letters represent statistical differences ($P < 0.05$). Numbers given in each bar indicate the number of oysters measured.

Clearance rate results expressed by individual (CRi) and standardized to an oyster of 1 g of dry meat weight (CRw) are also presented in Table 2; overall, clearance rate was significantly affected by the interaction of temperature, salinity, and population (CRw L h^{-1} g^{-1}: $P < 0.012$; CRi L h^{-1}: $P < 0.013$), with lower rates at 10°C compared with 20 °C and 30 °C, and higher clearance rates of New Brunswick oysters than Louisiana oysters only at 30 °C and a salinity of 15.

3.2. Valve opening

Time series plots of gape angle (°) revealed a strong rhythm in valve opening (Fig. 3A). There was a tendency for oysters to exhibit maximal gaping during the evening and late night hours and to close their valves

Table 1
Results of three-factor ANOVA analyses used to test the effects of temperature, salinity and population on *Crassostrea virginica* clearance rate, percentage time open, gape angle, oxygen consumption rate and condition index. Clearance rate was standardized by gill area (CRg) and oxygen consumption rate was standardized to an oyster of 1 g of dry meat weight (OCRw).

Source	d.f.	Clearance rate (CRg)	Percentage time open	Gape angle	Oxygen consumption rate (OCRw)	Condition index
Temperature (T)	2	< 0.001	0.007	0.989	< 0.001	0.010
Salinity (S)	1	0.898	0.654	0.622	0.236	0.291
Population (P)	1	0.182	0.296	0.002	0.037	< 0.001
T × S	2	0.233	0.465	0.851	0.437	0.884
T × P	2	0.171	0.084	0.393	0.811	0.353
S × P	1	0.490	0.476	0.063	0.560	0.594
T × S × P	2	0.001	0.722	0.578	0.780	0.115

Table 2
Shell height, dry meat weight, gill area, and clearance rate measurements expressed by individual (CRi) and standardized to 1 g of dry meat weight (CRw) for New Brunswick (NB) and Louisiana (LA) oysters at the three temperatures and two salinities tested. Data presented as mean ± standard deviation. Different letters represent statistical differences ($P < 0.05$).

Temperature (°C)	Salinity	Pop	N	Shell height (mm)	Dry meat (g)	Gill area (cm^2)	CRi ($L h^{-1}$)	CRw ($L h^{-1} g^{-1}$)
10	15	NB	4	75.7 ± 16.8	0.5 ± 0.2	2.7 ± 1.3	0.36 ± 0.17^{c}	0.54 ± 0.18^{d}
		LA	3	67.9 ± 3.9	0.7 ± 0.5	1.4 ± 0.8	0.43 ± 0.19^{c}	0.58 ± 0.04^{d}
	25	NB	3	74.1 ± 0.8	0.7 ± 0.1	2.5 ± 0.5	0.53 ± 0.13^{c}	0.67 ± 0.16^{d}
		LA	3	74.7 ± 8.4	1.5 ± 0.9	2.1 ± 0.6	0.22 ± 0.05^{c}	0.20 ± 0.16^{d}
20	15	NB	8	71.4 ± 4.2	0.4 ± 0.3	2.2 ± 0.7	2.16 ± 1.56^{ab}	3.94 ± 2.30^{abc}
		LA	7	71.1 ± 6.1	1.2 ± 0.4	1.7 ± 0.5	2.95 ± 1.53^{ab}	2.74 ± 1.30^{c}
	25	NB	12	69.7 ± 4.3	0.5 ± 0.2	2.1 ± 0.6	2.54 ± 1.40^{ab}	4.07 ± 2.07^{bc}
		LA	8	69.7 ± 4.2	1.0 ± 0.4	1.8 ± 0.5	4.19 ± 2.19^{ab}	4.45 ± 2.00^{bc}
30	15	NB	15	70.4 ± 5.1	0.5 ± 0.2	2.4 ± 0.7	5.15 ± 2.69^{a}	8.69 ± 3.77^{a}
		LA	16	69.0 ± 7.8	0.8 ± 0.5	1.7 ± 0.8	2.07 ± 1.25^{b}	2.72 ± 2.64^{c}
	25	NB	15	70.5 ± 5.8	0.5 ± 0.2	2.3 ± 0.4	3.77 ± 3.12^{ab}	6.50 ± 3.29^{ab}
		LA	10	68.7 ± 5.9	0.9 ± 0.5	1.5 ± 0.6	3.93 ± 1.25^{ab}	4.65 ± 1.78^{abc}

Letters represent homogeneous sub-groups.

during daylight hours. Cross-correlation analysis indicated that mean gaping lagged mean fluorescence by approximately one hour (Fig. 3B). Given the apparent influence of food levels on behavior further investigation of the dataset was constrained to an evening period (20 − 23h) when holding conditions were presumably closer to natural conditions given the abundant food level. For this period, oysters had their valves opened 74.3 ± 31.4% of the time ($N = 38$).

The percentage of time oysters had their valves opened was

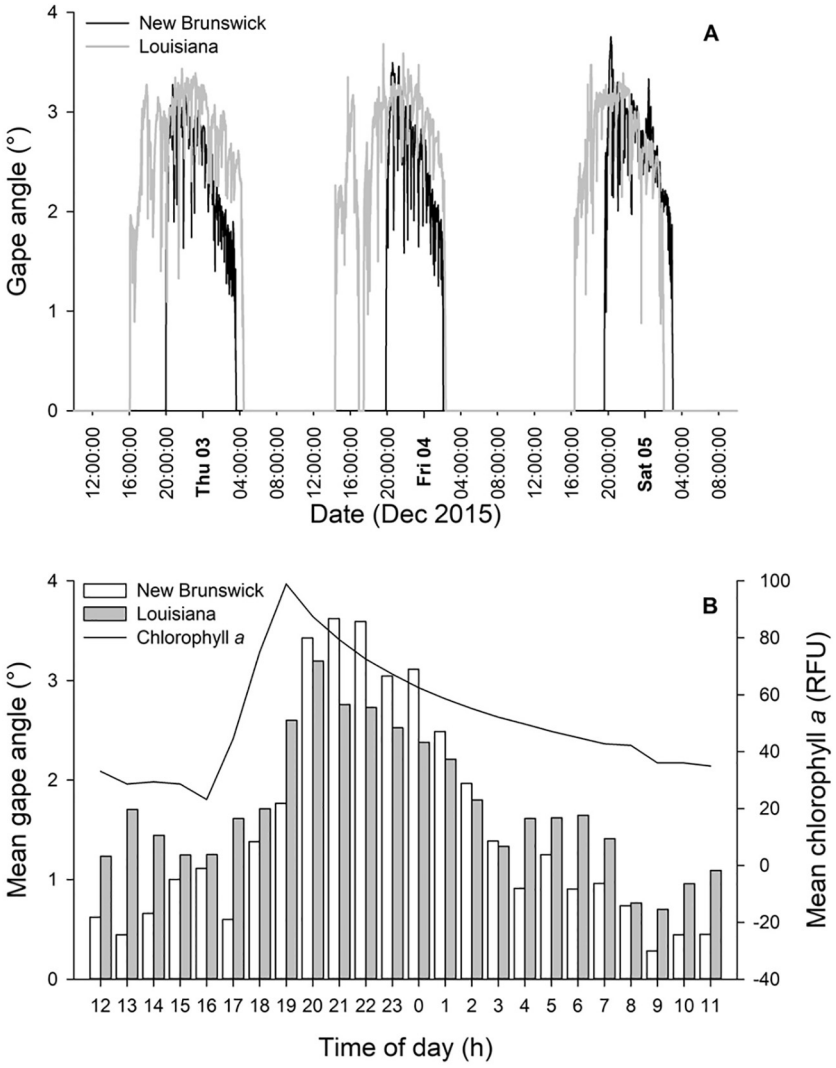

Fig. 3. Time-series plots showing valve opening on two populations of *Crassostrea virginica*. A) Examples of gaping behavior exhibited by a single New Brunswick oyster (dark line) and a single Louisiana oyster (grey line). B) Mean gaping and mean chlorophyll *a* fluorescence as a function of time-of-day for New Brunswick ($N = 19$ oysters) and Louisiana ($N = 21$ oysters).

Table 3
Shell height, dry meat weight, and oxygen consumption rate measurements expressed by individual (OCRi) and standardized to 1 g of dry meat weight (OCRw) for New Brunswick (NB) and Louisiana (LA) oysters at the three temperatures and two salinities tested.

Temperature (°C)	Salinity	Pop	N	Shell height (mm)	Dry meat (g)	OCRi (mg O_2 h^{-1})	OCRw (mg O_2 h^{-1} g^{-1})
10	15	NB	4	74.4 ± 5.7	0.8 ± 0.4	0.59 ± 0.39	0.68 ± 0.33
		LA	6	77.3 ± 5.4	1.7 ± 0.5	0.78 ± 0.36	0.55 ± 0.22
	25	NB	3	71.1 ± 3.5	0.4 ± 0.1	0.46 ± 0.06	0.81 ± 0.10
		LA	6	77.3 ± 7.3	1.2 ± 0.5	0.50 ± 0.30	0.50 ± 0.36
20	15	NB	10	72.1 ± 6.1	0.5 ± 0.2	0.69 ± 0.24	1.08 ± 0.34
		LA	5	67.8 ± 4.1	0.8 ± 0.1	0.82 ± 0.25	0.94 ± 0.29
	25	NB	8	74.9 ± 4.0	0.7 ± 0.2	0.86 ± 0.20	1.12 ± 0.27
		LA	6	71.6 ± 7.1	1.2 ± 0.5	0.98 ± 0.41	0.90 ± 0.22
30	15	NB	3	76.0 ± 7.1	0.3 ± 0.1	1.09 ± 0.26	2.09 ± 0.43
		LA	8	74.7 ± 4.6	1.0 ± 0.3	1.86 ± 0.77	1.90 ± 0.76
	25	NB	4	74.4 ± 6.6	0.4 ± 0.1	1.37 ± 0.13	2.55 ± 0.69
		LA	3	73.1 ± 6.0	1.0 ± 0.5	2.30 ± 0.88	2.33 ± 0.51

Data presented as mean ± standard deviation.

significantly affected by temperature (Table 1). Oysters at 20 °C (91.0 ± 18.5%, $N = 16$) had their valves opened a greater percentage of the time compared to oysters at 10 °C (64.4 ± 38.0%, $N = 12$) and 30 °C (61.4 ± 7.9%, $N = 10$). Salinity and population had no significant effect on the percentage of time open. Gape angle ranged between 0° (valve closed) and 9.7° (valve fully-opened) and was significantly affected by oyster population (Table 1). Louisiana oysters tended to exhibit restricted gaping (3.6 ± 0.8°, $N = 20$) compared to New Brunswick oysters (4.6 ± 1.1°, $N = 18$). Salinity and temperature had no significant effect on gape angle.

3.3. Oxygen consumption rate

Weight-standardized oxygen consumption rate was significantly affected by temperature and population (Tables 1 & 3). OCRw significantly increased from 10 °C (0.60 ± 0.28 mg O_2 h^{-1} g^{-1}, $N = 19$) to 20 °C (0.99 ± 0.29 mg O_2 h^{-1} g^{-1}, $N = 29$) and again at 30 °C (2.18 ± 0.67 mg O_2 h^{-1} g^{-1}, N = 18). Both populations responded similarly to temperature, but overall New Brunswick oysters (1.29 ± 0.70 mg O_2 h^{-1} g^{-1}, $N = 32$) had a significantly higher OCRw than Louisiana oysters (1.06 ± 0.78 mg O_2 h^{-1} g^{-1}, $N = 34$). Differences between salinities were not observed (15: 1.11 ± 0.70 mg O_2 h^{-1} g^{-1}, $N = 36$; 25: 1.24 ± 0.80 mg O_2 h^{-1} g^{-1}, $N = 30$).

Within the range of temperature and salinity tested in this study, only temperature and its quadratic had significant effects on predicting oxygen consumption rate, resulting in the following equation OCRw (mg O_2 h^{-1} g^{-1}) = 1.2804–0.0998 temperature + 0.0045 temperature^2, $r^2 = 0.72$, $P < 0.0001$ for New Brunswick oysters and OCRw (mg O_2 h^{-1} g^{-1}) = 0.8501–0.0678 temperature + 0.036 temperature^2, $r^2 = 0.68$, $P < 0.0001$ for Louisiana oysters (Fig. 4).

A 10 °C increase in water temperature over the range of 10–20 °C increased OCRw of New Brunswick oysters by approximately 1.5 (Q_{10} salinity of 15 = 1.58, Q_{10} salinity of 25 = 1.38) and 1.75 in Louisiana oysters (Q_{10} salinity of 15 = 1.71, Q_{10} salinity of 25 = 1.8). A 10 °C increase in water temperature over the range of 20–30 °C increased OCRw of New Brunswick oysters by approximately 2.10 (Q_{10} salinity of 15 = 1.94, Q_{10} salinity of 25 = 2.28) and 2.30 in Louisiana oysters (Q_{10} salinity of 15 = 2.02, Q_{10} salinity of 25 = 2.6).

3.4. Condition index, size, and shell shape

Condition index at the end of the experiment was significantly affected by temperature and population (Table 1). Condition index of oysters at 30 °C (5.4 ± 2.5, $N = 64$) was significantly lower than at 10 °C (6.3 ± 2.7, $N = 37$) and 20 °C (6.7 ± 2.9, $N = 45$) and the condition of Louisiana oysters (7.7 ± 2.4, $N = 77$) was significantly greater than in New Brunswick oysters (4.1 ± 1.4, $N = 69$). The higher condition index of the Louisiana oysters was due to their significantly

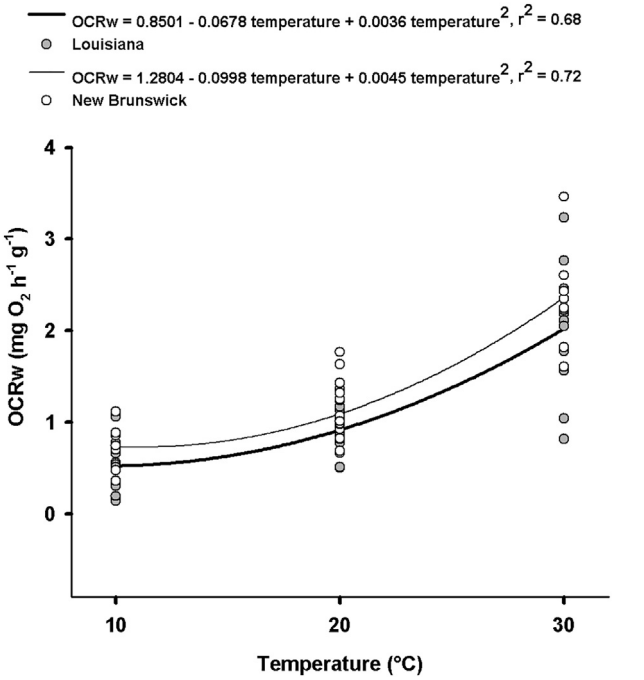

Fig. 4. Weight-standardized oxygen consumption rate (OCRw) versus temperature in New Brunswick (white circles) and Louisiana (grey circles) oysters, and polynomial regressions for both populations.

greater dry meat weight ($P < 0.001$, LA: 1.0 ± 0.5 g, $N = 79$; NB: 0.5 ± 0.2 g, $N = 72$) relative to their filled shell cavity weight ($P = 0.222$, LA: 11.8 ± 4.3 g, N = 79; NB: 10.8 ± 2.4 g, N = 72).

No differences ($P = 0.219$) in shell height were detected between New Brunswick (70.9 ± 6.4 mm, $N = 60$) and Louisiana (69.5 ± 6.3 mm, $N = 57$) oysters; however, New Brunswick oysters (2.29 ± 0.65 cm^2, N = 57) had significantly ($P < 0.001$) larger gill area than Louisiana oysters (1.72 ± 0.65 cm^2, $N = 55$).

Oyster cup and fan ratio was higher in Louisiana (0.37 ± 0.1 and 0.71 ± 0.1, $N = 80$) than in New Brunswick (0.29 ± 0.1 and 0.66 ± 0.1, $N = 71$) oysters. The ratio between dry shell weight and shell height was also higher in Louisiana (0.65 ± 0.29, N = 80) than New Brunswick (0.37 ± 0.1, N = 71) oysters.

4. Discussion

The effect of the combination of three temperatures (10, 20, and 30 °C) and 2 salinities (15 and 25) on the clearance rate, valve opening, and oxygen consumption rate of *C. virginica* from Louisiana and New

Brunswick were compared in this study. No differences in clearance rate (standardized to gill area) or valve opening duration was found between the two latitudinally-distant populations; however, the oxygen consumption rate of northerly New Brunswick oysters was slightly greater than that of Louisiana oysters. Results revealed a tolerance of the species to the conditions tested and the physiological plasticity of two populations originating from different environmental conditions. While in the estuaries of Gulf of St. Lawrence mean annual water temperature and salinity are about 8 °C and 28 (Comeau et al., 2012; Fisheries and Oceans Canada, unpublished data), in southeast Louisiana mean water temperature is about 23 °C and mean salinity is 11 (Casas et al., 2015, 2017; Leonhardt et al., 2017; Lowe et al., 2017). The extent to which an organism's physiology determines its geographic range remains an unresolved problem in integrative biology, but tolerance ranges and physiological plasticity may positively be related with range extension (Bozinovic and Naya, 2015).

Temperature had the greatest influence in *C. virginica* clearance rate, with a reduction in rates at 10 °C compared with rates at 20 °C and 30 °C. Loosanoff (1958) measured the volume of water passing through the gills of *C. virginica* or pumping rates between 0 °C and 38 °C, and observed a low rate below 8 °C, a steadily increasing rate from 8 °C to 16 °C, no marked differences between 16 °C and 28 °C, a further increase between 28 °C and 32 °C, and finally a decrease in pumping rate beyond 34 °C. Temperature affects both the rates of biological and physiological processes (i.e., ciliary beat frequency, reaction rates, enzyme efficiencies) and the physical properties of the water (i.e., water viscosity), and it has been estimated that the increase in water viscosity at low temperatures could account for about a 50% decrease in filtration rate (reviewed by Bayne, 2017). Under optimal range of temperature (i.e. 20 °C and 30 °C in this study or 17 °C and 27 °C in Casas et al., 2018) clearance rate is similar and independent of temperature.

Although a pattern of intraspecific variation in growth rate along latitudinal gradients has been found in many ectotherm species, the underlying physiological mechanisms responsible for these differences are not well understood (Levinton, 1983; Conover and Present, 1990; Dittman et al., 1998; Angilletta Jr et al., 2004). Studies in several fish species found that higher food intake and food conversion efficiency in northern populations likely explained observed growth rate differences (Present and Conover, 1992; Jonassen et al., 2000). In contrast, there is limited information on food ingestion by bivalve populations from different latitudes. Rao (1953) studied the pumping rate of the California mussel, *Mytilus californianus*, from three latitudes (34 to 48°N) and found greater pumping rates at any given temperature (9, 16, and 20 °C) in mussels from higher latitudes than in mussels of corresponding weight from lower latitudes. However, results were inconclusive because mussels from northern latitudes had also lower meat weight, and shell height or gill area was not reported and used for standardization. Clearance and pumping rate should be expressed relative to gill area or shell height units to recognize the direct relationship between gill area and pumping rate (Meyhöfer, 1985; Riisgård, 1988) and to avoid standardization errors related to the condition of the animals compared (Filgueira et al., 2008; Cranford et al., 2011). In our study, although oysters were the same size, given the geographic distance and concomitant environmental differences, oysters were morphologically very different. For example, the gill area was larger in New Brunswick oysters and dry meat was greater in Louisiana oysters. Gill size in bivalves relates to particle concentration, food quantity and quality, and adapts to fit environmental conditions (Theisen, 1982; Franz, 1993; Payne et al., 1995; Dutertre et al., 2007). Accordingly, in conditions of elevated particle concentration, as is the case of Louisiana estuaries, smaller gills and larger labial palps would be advantageous to increase the efficiency of particle selection and to avoid saturation of other feeding processes. In contrast, when food is scarce or there are limited periods of availability, as for example in the Gulf of St. Lawrence, larger gills could be beneficial. Gill area to dry meat weight ratio was more than double in New Brunswick (5.7) than Louisiana (2.2) oysters, and this ratio can likewise be affected by multiple factors, most of them differing among latitudinal distant locations, for example food quality and quantity, gametogenic cycle, feeding time, and age (Worrall et al., 1983; Franz, 1993; Barrillé et al., 2000). Some studies have shown how differences in the gill area to body weight among populations of the same species were largely environmental and a demonstration of the plasticity of the physiological response (Worrall and Widdows, 1983; Worrall et al., 1983; Essink et al., 1989). Despite the morphological differences (i.e., gill area and dry meat weight) between both oyster populations, differences in their clearance rates (CRi, CRg, CRw) were not found. Accordingly, the similar clearance rate by gill area between populations when measured under the same experimental temperature, salinity and food conditions, suggests that environmental conditions, and not the origin of the oysters, are the main drivers of clearance rate.

Oysters tended to exhibit maximal gaping during periods of darkness and to close their valves during daylight hours. This circadian rhythmicity was likely driven to a large extent by the batch feeding protocol, in which oysters were given a fixed ration at the end of each work day. As observed previously in other bivalves (Higgins, 1980; Riisgård et al., 2003), the sudden increase in the availability of algae likely prompted the oysters to open their valves and graze throughout the evening and most of the night. Depending on the temperature, oysters had their valves opened 61% to 91% of the time following the addition of food into the holding tanks. Similarly, oysters maintained under natural conditions were reportedly opened 69% to 97% of the time (Comeau et al., 2012). In our experiment, temperature significantly affected behavior; enhanced propensity for openness in the evening was observed at 20 °C, suggesting an enhanced response to exogenous factors (e.g., food) at this particular temperature. In contrast, the lowered propensity for valve openness detected at 10 °C and 30 °C may be indicative of stressful environments for the species. Oysters can isolate themselves by closing their valves when exposed to unfavorable environmental conditions (Casas et al., 2018). The origin (population) of oysters had only a significant impact on gaping angles, with the Louisiana oysters displaying restricted gaping angles compared to New Brunswick oysters. The extent to which valves can gape maximally depends largely on the shape and size of the beaks and the level of interumbonal shell growth (Stasek, 1963; Galtsoff, 1964). For example, in oysters with proportionally large space outside the ligament between the beaks, valve can gape widely, whereas in oysters with close-set beaks, movement of the valves on the hinge is severely limited regardless of the degree of relaxation of the adductor muscle. Valves shape and thickness in *C. virginica* can vary within populations and in different habitats suggesting a high degree of plasticity of the shell formation in response to environmental and genetic factors (Carriker, 1996; Lord and Whitlatch, 2014). Oysters from Louisiana were rounder and wider than New Brunswick oysters and had heavier shells (Fig. 1). Heavier and more convex shells have been also reported in bivalves from lower latitudes (Wilbur and Gaffney, 1997; Lord and Whitlatch, 2014), suggesting elevated shell deposition rates at higher temperatures (Lord and Whitlatch, 2014), and a protection mechanism from predation by becoming thicker, larger, and more difficult to crush even at a smaller size (Wilbur and Gaffney, 1997). Thicker shells with close-set beaks could be the reason for the narrower valve opening in Louisiana oysters; however more studies in the form of the beaks and how they are affected by environmental and genetic factors are needed.

Temperature exerted a major effect on oxygen consumption rate, which gradually increased from 10 °C to 30 °C as described in previous studies (Schumway and Koehn, 1982), with no evidence of suppressed rate or thermal breakpoint at 30 °C, but with an impact on the condition index. *Crassostrea virginica* can live at water temperatures above 30 °C (Leonhardt et al., 2017; Casas et al., 2017) and with seawater temperatures predicted to increase (IPCC, 2014), there is a need to study oxygen consumption rates above 30 °C and identify the temperature breakpoint for the species. In marine invertebrates, warm temperatures increase oxygen demand which is fulfilled by an increase in ventilation

and heart rate up to a critical breakpoint or threshold temperature when oxygen demand is greater than the organism's capacity to uptake and delivery oxygen to the tissues, leading to anaerobic metabolism and progressive functional and energetic failure (Pörtner, 2001; Jansen et al., 2007). Oxygen consumption rate in New Brunswick oysters was significantly greater than in Louisiana oysters. Despite the differences between populations, the oxygen consumption rate-temperature curve in both populations were parallel, indicating a similar degree of sensitivity to temperature (Fig. 4), and that other internal drivers such as growth or reproductive status, and not latitudinal compensation, are the main drivers of the different rates. Short-term changes in temperature (within days to weeks) typically affect the metabolic rate (or oxygen consumption rate) vs temperature curve by changing the y-intercept of the linear regression, whereas adaptive long-term changes (intergenerations, years) will involve a change in the slope instead of the intercept (Terblanche et al., 2009). Lord and Whitlatch (2014) measured the oxygen consumption rates of *C. virginica* populations distributed along the Atlantic coast from New Brunswick to Florida and found that their rates fell along a similar temperature-oxygen consumption rate curve. They found no indications of compensation to latitude, with oysters showing a great physiological plasticity to temperature and seasonal changes. Similarly, Clarke (1993) concluded that latitudinal metabolic compensation does not exist as there is no evidence for any compensatory adjustments in the metabolic rate of marine organisms related to the different temperatures at various latitudes. Furthermore, patterns of metabolic rate that are usually explained in terms of acclimation to seasonal or latitudinal changes in temperature are related primarily to other factors, i.e. variations in growth and reproduction (Clarke, 1993).

Oxygen consumption increased with temperature at a higher rate between 20 °C and 30 °C (Q_{10} ranged between 1.9 and 2.6) than between 10 °C and 20 °C (Q_{10} between 1.4 and 1.8), regardless of salinity. Schumway and Koehn (1982) observed similar values and patterns across temperatures at the salinity of 28, but they found a higher Q_{10} at a salinity of 14 than at 28. Contrary to our observations, Dame (1972) found a decrease in the Q_{10} values with an increase in temperature (10 °C to 30 °C) but the oxygen consumption measurements at the different temperatures were done at different times of the year (January, July, November). In addition to temperature, seasonal differences in metabolism (i.e. variations in growth and reproduction cost) were also affecting the oxygen consumption rates in his study. Q_{10} values stand around two within the normal ecological temperature range of the organism, and values lower than one only occur beyond the breakpoint temperature when the metabolic rate decreases with increasing temperature (Pörtner, 2001; Jansen et al., 2007; Bayne, 2017). In our study, both populations have Q_{10} above 1, showing no signs of thermal sensitivity in their oxygen consumption rate.

New Brunswick and Louisiana oyster populations showed physiological plasticity in their response to salinity (15 and 25). No physiological differences were found between salinities in both populations, when Louisiana oysters came from low-intermediate estuaries with a yearly mean salinity of 11, while New Brunswick oysters originated from an intermediate-high salinity estuary with a mean salinity of 28. Since the species physiological optimum is probably between 15 and 25, both populations performed at their highest rates in the conditions assayed. The response of oysters to more extreme salinities (i.e. lower than 15), although never experienced by New Brunswick oysters, would be interesting to investigate as oyster populations from Louisiana regularly face values as low as 1 (Lowe et al., 2017). Also, because oysters were maintained at the tested salinities for more than three weeks before the measurements were done, the metabolic costs of adapting to the new salinities were not captured in the study.

5. Conclusion

New Brunswick and Louisiana oyster populations showed tolerance to a broad range of temperatures (10–30 °C in this study) and physiological plasticity, with no evidence for physiological compensation to latitudinal differences. No differences in clearance rate and valve opening duration were found between populations. New Brunswick oysters had larger gills, but differences in clearance rate with Louisiana oysters were not found. Oxygen consumption rate was higher in New Brunswick compared to Louisiana oysters, however both populations responded similarly to temperature, with parallel temperature-oxygen consumption rate curves, suggesting that these differences could result from a differential internal status between populations and not from latitudinal compensation. Oxygen consumption rate increased with temperature in both populations without indications of a thermal breakpoint at 30 °C. Although a wide temperature range was tested, the logistic limitations of the study didn't allow us to examine the full range of temperatures experimented by the species (i.e. 0 °C, 32 °C) to possibly detect differences between both populations at more extreme conditions. It is clear that more research is needed to advance our knowledge on population physiology and understand its links with distribution range and predict its response to climate change.

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Competing interests

The authors declare no competing or financial interests.

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